MycoKeys

MycoKeys 122: 223–236 (2025)
DOI: 10.3897/mycokeys.122.161471

Research Article

Two new species of *Meliola* (Meliolales, Sordariomycetes) from Guizhou Province, China

Yuzhe Feng[1,2,3], Yuwei Liu[1], Sinang Hongsanan[4], Entaj Tarafder[1], Xiaolu Deng[1,2,3], Tian Yang[1,2,3], Jipeng Sun[1,2], Ting-Chi Wen[3,5,6], Xiang-Yu Zeng[1,2,3], Fengquan Liu[1]

1 *Department of Plant Pathology, College of Agriculture, Guizhou University, Guiyang, 550025 China*
2 *Guizhou Key Laboratory of Agricultural Microbiology, Guiyang, 550025 China*
3 *Institute of Edible Mushrooms, Guizhou University, Guiyang, 550025 China*
4 *Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, Shenzhen 518060, China*
5 *State Key Laboratory of Green Pesticide, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang 550025, China*
6 *Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, Guiyang 550025, China*
Corresponding authors: Xiang-Yu Zeng (xyzeng3@gzu.edu.cn), Fengquan Liu (fqliu20011@sina.com)

Abstract

Meliola is a genus of specialized parasites that grow on the surface of living plant leaves and fruits, potentially impairing host growth, reducing fruit quality and crop yield, and ultimately causing economic losses. In this study, two new species of *Meliola* from Guizhou Province, China, were identified based on morphological characteristics and multi-gene phylogenetic analyses (nrITS, nrLSU, and nrSSU) using maximum likelihood (ML) and Bayesian inference (BI) to clarify their taxonomic placement. The new species, *Meliola liboense* and *M. lindericola*, were both found colonizing leaves of *Lindera*. Detailed descriptions, illustrations, and a phylogenetic tree showing the phylogenetic positions of the two new species are provided. These findings enrich the taxonomic diversity of *Meliola* and contribute valuable morphological and molecular data to improve species delimitation and classification within Meliolaceae.

Key words: Black mildews, fungal diversity, new species, phylogenetic analysis, taxonomy

OPEN ACCESS

This article is part of:
Exploring the Hidden Fungal Diversity: Biodiversity, Taxonomy, and Phylogeny of Saprobic Fungi
Edited by Samantha C. Karunarathna, Danushka Sandaruwan Tennakoon, Ajay Kumar Gautam

Academic editor:
Danushka Sandaruwan Tennakoon
Received: 8 June 2025
Accepted: 16 August 2025
Published: 15 September 2025

Citation: Feng Y, Liu Y, Hongsanan S, Tarafder E, Deng X, Yang T, Sun J, Wen T-C, Zeng X-Y, Liu F (2025) Two new species of *Meliola* (Meliolales, Sordariomycetes) from Guizhou Province, China. MycoKeys 122: 223–236. https://doi.org/10.3897/mycokeys.122.161471

Copyright: © Yuzhe Feng et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Introduction

Meliola is the largest and the type genus of the family Meliolaceae (Meliolales, Sordariomycetes), comprising 1,765 species (Khan et al. 2025) and accounting for more than 70% of species within the family (Zeng et al. 2022). Species of *Meliola* are obligately biotrophic phytopathogens that form black colonies on living plant surfaces. They establish parasitic relationships with their host plants by forming appressoria on the surface of host epidermal cells, which facilitate adhesion and penetration. From these appressoria, haustoria subsequently develop and extend into the host epidermis or mesophyll cells to absorb nutrients, resulting in a reduction of chlorophyll, starches, sugars, proteins, and amino acids, but without causing pathogenic damage (Hansford 1961; Old et al. 2003; Rodríguez Justavino and Piepenbring 2007; Hongsanan et al. 2015). While exhibiting high host specificity

(infecting nearly 200 plant families, particularly Fabaceae), their exact host range requires molecular validation due to potential taxonomic ambiguities (Hansford 1961; Hongsanan et al. 2015). Members of *Meliola* are characterized by branched, dark brown, superficial mycelium with phialides and 2-celled appressoria; dark brown, flattened to subglobose ascomata with 2–4-spored asci; and cylindrical, dark brown ascospores with 3–4 septa. They differ from other genera in Meliolaceae by having hyphal setae on the mycelium (Goos 1974) and by lacking vermiform appendages and setae on the surface of perithecia (Hansford 1961). The genus *Meliola* was first established by Fries in 1825. The description was later revised by Bornet in 1851, with *Meliola psidii* Fr. (Fries 1830) designated as the type species. Gaillard (1892) and Beeli (1920) provided comprehensive studies on the genus, notably introducing digital formulae to facilitate comparisons among genera and species. Subsequently, the taxonomy of *Meliola* was gradually refined through studies focusing on morphological characteristics and systematic placement (Stevens 1927, 1928; Hansford 1961, 1963; Hosagoudar 2008, 2013). As taxonomic understanding progressed, species lacking hyphal setae were reassigned to newly established genera, and these taxonomic revisions significantly contributed to delineating the genus *Meliola* and its morphological characteristics (Stevens 1927, 1928; Hansford 1961). While early classifications placed *Meliola* within Perisporiales or Ascoloculares (Stevens 1927; Nannfeldt 1932), it is now recognized under the order Meliolales, class Sordariomycetes (Lumbsch and Huhndorf 2010).

Because of their biotrophic lifestyles, species of *Meliola* have yet to be grown in culture. Although ascospore germination was successfully induced by Thite (1975) and Goos (1978), subsequent hyphal growth was extremely slow and ultimately ceased, preventing the establishment of in vitro cultures. Molecular data for species of *Meliola* have therefore been obtained by directly extracting DNA from ascomata. However, this method is often complicated by the presence of hyperparasites (Bermúdez-Cova et al. 2022, 2023) and contaminating microorganisms on the colony surface, which can compromise sequencing quality by introducing non-target DNA and reducing the purity of the extracted genetic material. Owing to the difficulties in isolation and molecular sequencing, available molecular data for this genus remain limited. Although the genus *Meliola* currently comprises 2,365 species and 701 varieties, molecular data are available for only 23 species with reliable taxonomic support in GenBank, including the two newly described in this study (accessed 16 April 2025). The taxonomic framework proposed by Hansford (1961), which classifies species primarily based on host specificity and morphological characteristics, is still widely adopted in recent studies (Hongsanan et al. 2015; Zeng et al. 2017, 2022). Given the current paucity of molecular data, species delineation remains largely contingent upon morphological analysis, particularly comparative studies with previously described congeners associated with identical or phylogenetically proximate host plants. Diagnostic morphological traits include the size and shape of perithecia, haustoria, spores, and setae; the arrangement of attachment cells and phialides; and variation in haustorial structure (Hongsanan et al. 2015; Zeng et al. 2022). While a few lineages have received preliminary support from molecular analyses, the overall phylogenetic framework of the genus remains largely morphology-based, lacking a comprehensive and robust molecular systematics foundation.

In this study, we introduce two new species (*M. liboense* and *M. lindericola*) of Meliolaceae, collected from living plant leaves in Guizhou, China. Morphological

observations and phylogenetic analyses were conducted to clarify the classification of these species and their phylogenetic relationships with closely related taxa. Detailed descriptions of the morphological features of these species, along with their molecular characterization, are provided.

Materials and methods

Sample collection and morphological observation

Samples of leaves infected with black mildews were collected from Libo County, Guizhou Province, China, and brought to the laboratory in paper envelopes, then stored in a dry environment. Specimens were examined using a stereomicroscope (Keyence VHX–7000 digital microscope, Japan) to observe and photograph macroscopic features, including colony structure, color, and distribution of ascomata. Microscopic observations and photomicrographs were made from materials mounted in 60% lactic acid using a compound light microscope (Zeiss Axioscope 5, Germany) equipped with an AxioCam 208 color camera and interference contrast optics. Images were captured to document the morphology, color, size, and other characteristics of microstructures such as hyphae, appressoria, setae, ascomata, spores, and phialides. Measurements were taken using the ZEN2 (blue edition) software, and all images used in figures were processed with Adobe Photoshop (version 2022). Study specimens were deposited in the Herbarium of IFRD (International Fungal Research & Development Centre; Institute of Highland Forest Science, Chinese Academy of Forestry, Kunming, China). Index Fungorum numbers were obtained by submitting the species information via the official online registration platform (Index Fungorum 2025).

Host plant identification

A preliminary morphological identification of the host plant was performed, followed by molecular biological identification. Morphological identifications were confirmed through consultation with taxonomic experts. Total genomic plant DNA was extracted using a Plant gDNA maxi kit (Biomiga, San Diego, California, USA) in accordance with the manufacturer's instructions. The partial *rbcL* gene was amplified using the primer pair SI_For and SI_Rev, which were developed by Kress et al. (2009). The 5.8S rDNA along with the internal transcribed spacer (ITS) was amplified with the primer pair ITS1 and ITS4 (White et al. 1990).

DNA extraction, PCR amplification, and sequencing

Total genomic fungal DNA was directly extracted from ascomata following the protocol described by Zeng et al. (2018). The 5.8S ribosomal RNA along with the internal transcribed spacer (ITS) was amplified with the primer pair ITS1 and ITS4 (White et al. 1990). The partial large subunit ribosomal RNA (LSU) was amplified with the primer pair LR0R and LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1994). The partial small subunit ribosomal RNA (SSU) was amplified with the primer pair NS1 and NS4 (White et al. 1990). Polymerase chain reactions (PCRs) were performed in a 20 µL reaction mixture containing 17 µL of Golden-Star T6 Super PCR Mix (1.1×), 1 µL of DNA template, and 1 µL each of forward

Table 1. The PCR conditions and the primers used in this study.

Locus	Primers	Sequence (5′–3′)	PCR cycles	References
ITS	ITS1	TCCGTAGGTGAACCTGCGG	(95°C: 30s, 54 °C:30s, 72°C:60s) × 35 cycles	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC		
LSU	LR0R	ACCCGCTGAACTTAAGC	(94°C: 30s, 51°C: 50s, 72°C:60s) × 35 cycles	Rehner and Samuels (1994)
	LR5	TCCTGAGGGAAACTTCG		Vilgalys and Hester (1990)
SSU	NS1	GTAGTCATATGCTTGTCTC	(95°C: 30s, 51 °C: 50s, 72°C: 60s) × 35 cycles	White et al. (1990)
	NS4	CTTCCGTCAATTCCTTTAAG		
RBCL–a	RBCLf	ATGTCACCACAAACAGAGACTAAAGC	(95°C: 30s, 50 °C: 50s, 72°C: 60s) × 35 cycles	Kress et al. (2009)
	RBCLr	GTAAAATCAAGTCCACCRCG		

and reverse primers (10 μM/μL). Amplifications were programmed for an initial denaturation step at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 54°C (ITS), 50 s at 50°C (LSU), 51°C (SSU), and 50°C (*rbcL*), and 90 s at 72°C, with a final elongation step of 10 min at 72°C. PCR amplification products were assayed via electrophoresis in 1% agarose. The PCR products were then sent to Tsingke Biotechnology Co., Ltd., Beijing, China. The list of primers and PCR conditions for each primer pair is provided in Table 1.

Phylogenetic analyses

New DNA sequences of each gene, generated from forward and reverse primers, were assembled using BioEdit v.7.2.5 (Hall 1999) to obtain consensus sequences. Phylogenetic analyses were conducted based on datasets including both reference DNA sequences and newly generated DNA sequences using OFPT (Zeng et al. 2023), following the protocol below. Datasets of each gene region were first independently aligned with the "FFT-NS-i" strategy (based on data size) by MAFFT (Katoh and Standley 2013) and trimmed manually. The best-fit nucleotide substitution models for each dataset were then selected according to the Bayesian information criterion (BIC) from 22 common DNA substitution models with rate heterogeneity, using ModelFinder (Kalyaanamoorthy et al. 2017). All datasets were subsequently concatenated with partition information for the following phylogenetic analyses. Maximum likelihood (ML) analysis with 1,000 replicates was performed using ultrafast bootstrap approximation (Hoang et al. 2017) with the SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010) implemented in IQ-TREE (Nguyen et al. 2015) The consensus tree was summarized based on the extended majority-rule criterion. Bayesian inference (BI) was performed with two parallel Metropolis-coupled runs (one "cold" chain and three heated chains) using MrBayes (Ronquist et al. 2012).

Results

Molecular phylogeny

The newly generated sequences were deposited in GenBank, and their accession numbers are provided in Table 2. Phylogenetic analyses for Meliolaceae were performed using ITS, LSU, and SSU loci. The concatenated dataset consisted of 33 strains, with *Sordaria fimicola* designated as the outgroup. The final alignment

comprised 2,026 concatenated characters, spanning positions 1–179 (ITS), 180–1,046 (LSU), and 1,047–2,026 (SSU). The combined dataset yielded a best-scoring tree with a final ML optimization likelihood value of −9724.306753. The phylogenetic information used for ML and BI analyses, including model selection and sequence characteristics, is detailed in Table 3. The topology of the tree from BI was similar to that obtained from ML. Given the similarity between the ML and BI topologies, only the ML tree is shown (Fig. 1).

Based on the ML and BI analyses, Meliolaceae consists of four generic lineages, represented by the genera *Meliola*, meliola-like, *Asteridiella*, and *Irenopsis*. The genera *Meliola* (20 representative sequences), *Irenopsis* (3), meliola-like (5), and *Asteridiella* (4) form a monophyletic group, with *Meliola* sister to *Irenopsis* and meliola-like sister to *Asteridiella*.

Table 2. GenBank accession numbers of DNA sequences used in this study.

Species	Specimen voucher	GenBank accession number			References
		SSU	ITS	LSU	
Asteridiella combreticola	MFLU 17-1041	NG_077427	NR_174812	MN747481	Zeng et al. (2022)
Asteridiella viticis	MFLU 19-1008	–	NR_174824	NG_079538	Marasinghe et al. (2020)
Asteridiella lozanellae	MP 3432	DQ508301	–	DQ508302	Rodríguez Justavino and Piepenbring (2007)
Asteridiella nitidae	ppMP 796	–	–	EF094839	Rodríguez Justavino et al. (2015)
Asteridiella obesa	VIC 31239	NG_065009	NR_120256	NG_057014	Pinho et al. (2012a)
Irenopsis crotonicola	MFLU 14-0078	NG_065668	KY554798	KY554798	Zeng et al. (2017)
Irenopsis vincensii	VIC 31752	KC618652	NR_154061	JX096807	Pinho et al. (2012a)
Irenopsis walsurae	MFLU 13-0621	NG_228705	NR_154075	KT021648	Zeng et al. (2022)
Meliola acacia	MFLU 16-0077	NG_077426	NR_174811	MN747479	Zeng et al. (2022)
Meliola aristolochiae-tagalae	MFLU 16-0088	MN747496	–	NG_079535	Zeng et al. (2022)
Meliola camporesii	MFLU 19-1005	–	NR_186987	NG_228823	Bhunjun et al. (2022)
Meliola centellae	VIC 31244	–	NR_137799	JQ734545	Pinho et al. (2012a)
Meliola citri-maximae	MFLU 14-0288	NG_070325	NR_185523	KX458474	Hyde et al. (2016)
Meliola clerodendricola	MFLU 13–0620	MN747486	KT021647	KT021647	Zeng et al. (2022)
Meliola clerodendri-infortunati	MFLU 13-0624	NG_070324	NR_160479	MF175626	Zeng et al. (2022)
Meliola jasmini-sambac	MFLU 17-1044	NG_077428	NR_174813	MN747482	Zeng et al. (2022)
Meliola lithocarpigena	MFLU 13-0628	NG_077423	NR_174809	MN747474	Zeng et al. (2022)
Meliola mucunicola	MFLU 15-0386	–	KT157534	KT157533	Hongsanan et al. (2015)
Meliola peruiferi	VIC 31249	–	–	NG_060294	Pinho et al. (2012b)
Meliola pistaciicola	MFLU 16-0070	NG_077425	MN747478	MN747478	Zeng et al. (2022)
Meliola pottsiae	MFLU 13-0631	NG_077424	NR_174810	MN747475	Zeng et al. (2022)
Meliola pseudosasae	MFLU 16-2136	–	–	KX845434	Hyde et al. (2016)
Meliola puerariae	MFLU 16-0087	–	–	NG_079534	Zeng et al. (2022)
Meliola schimigena	MFLU 17-1048	–	MN747484	MN747484	Zeng et al. (2022)
Meliola tamarindi	MFLU 14-0080	KY554797	KY554799	KY554799	Zeng et al. (2018)
Meliola telosmae	MFLU 14-0003	MK103390	MK103389	MK103389	Chethana et al. (2021)
Meliola thailandicum	MFLU 16-0067	MN747492	MN747476	MN788606	Hongsanan et al. (2015)
Meliola variaseta	DRJ 54	–	–	EF094840	Pinho et al. (2012a)
Meliola vernalis	VIC 31240	–	–	JX096808	Pinho et al. (2012a)
Meliola libosense	IFRD99008	PQ483902	PQ481147	PQ483770	This Study
Meliola libosense	IFRD900-9	PQ483899	PQ481145	PQ483767	This Study
Meliola libosense	IFRD900-10	PQ483901	PQ481146	PQ483769	This Study
Meliola lindericola	IFRD99007	PQ483900	PQ481148	PQ483768	This Study
Sordaria fimicola	AFTOL-ID 216	AY545724	DQ518178	AY545728	Muhammad et al. (2017)

Table 3. Summary of phylogenetic parameters used in ML and BI analyses, including the best-fit substitution models, number of nucleotide positions, conserved and variable sites, and other relevant characteristics for each gene region.

Information	Gene		
	ITS	LSU	SSU
Best fit substitution mode	F81+F+R5	GTR+F+I+G4	TIM2e+R2
Number of sequences	25	33	22
Number of characters	179	867	980
Number of constant sites	121 (representing 67.60% of all sites)	525 (representing 60.55% of all sites)	869 (representing 88.67% of all sites)
Number of parsimony informative sites	42	248	50
Number of distinct site patterns	63	387	100
Estimated base frequencies	A = 0.2912	A = 0.2484	equal frequencies
	C = 0.2416	C = 0.216	
	G = 0.2314	G = 0.3152	
	T = 0.2358	T = 0.2203	
Estimated substitution rates	A-C = 1.0000	A-C = 0.8300	A-C = 3.0548
	A-G = 1.0000	A-G = 5.4341	A-G = 6.8999
	A-T = 1.0000	A-T = 1.9822	A-T = 3.0548
	C-G = 1.0000	C-G = 0.3599	C-G = 1.0000
	C-T = 1.0000	C-T = 10.1670	C-T = 15.5297
	G-T = 1.0000	G-T = 1.0000	G-T = 1.0000

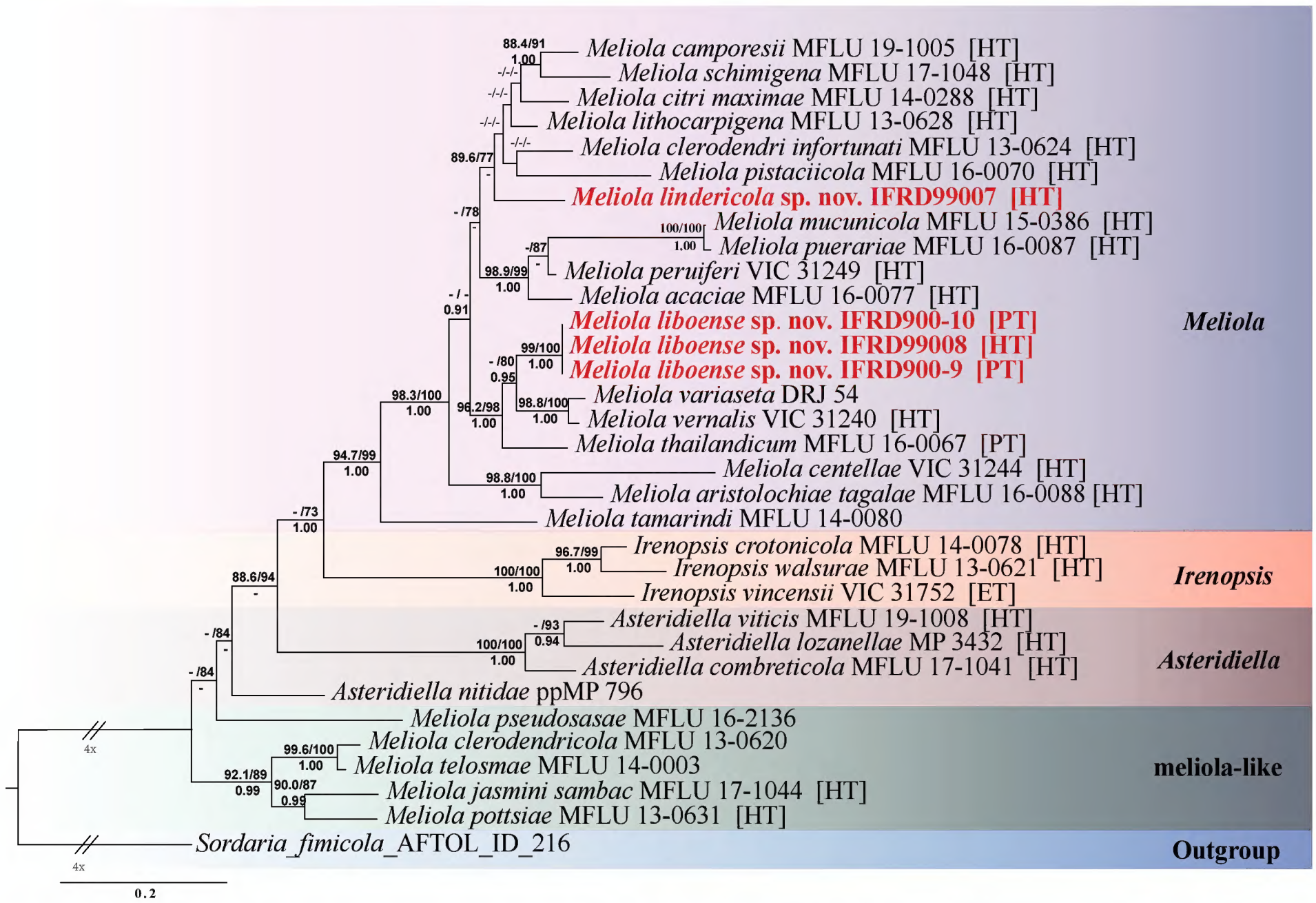
Meliola camporesii MFLU 19-1005 [HT]
Meliola schimigena MFLU 17-1048 [HT]
Meliola citri maximae MFLU 14-0288 [HT]
Meliola lithocarpigena MFLU 13-0628 [HT]
Meliola clerodendri infortunati MFLU 13-0624 [HT]
Meliola pistaciicola MFLU 16-0070 [HT]
Meliola lindericola sp. nov. IFRD99007 [HT]
Meliola mucunicola MFLU 15-0386 [HT]
Meliola puerariae MFLU 16-0087 [HT]
Meliola peruiferi VIC 31249 [HT]
Meliola acaciae MFLU 16-0077 [HT]
Meliola liboense sp. nov. IFRD900-10 [PT]
Meliola liboense sp. nov. IFRD99008 [HT]
Meliola liboense sp. nov. IFRD900-9 [PT]
Meliola variaseta DRJ 54
Meliola vernalis VIC 31240 [HT]
Meliola thailandicum MFLU 16-0067 [PT]
Meliola centellae VIC 31244 [HT]
Meliola aristolochiae tagalae MFLU 16-0088 [HT]
Meliola tamarindi MFLU 14-0080
Irenopsis crotonicola MFLU 14-0078 [HT]
Irenopsis walsurae MFLU 13-0621 [HT]
Irenopsis vincensii VIC 31752 [ET]
Asteridiella viticis MFLU 19-1008 [HT]
Asteridiella lozanellae MP 3432 [HT]
Asteridiella combreticola MFLU 17-1041 [HT]
Asteridiella nitidae ppMP 796
Meliola pseudosasae MFLU 16-2136
Meliola clerodendricola MFLU 13-0620
Meliola telosmae MFLU 14-0003
Meliola jasmini sambac MFLU 17-1044 [HT]
Meliola pottsiae MFLU 13-0631 [HT]
Sordaria_fimicola_AFTOL_ID_216
Meliola
Irenopsis
Asteridiella
meliola-like
Outgroup
4x
4x
0.2

Figure 1. Best-scoring ML tree based on combined LSU, SSU, and ITS sequence data of Meliolaceae, rooted by *Sordaria fimicola*. The SH-aLRT values greater than 80%, BS values greater than 70%, and PP values greater than 0.9 are shown above the branches. Sequences obtained from holotype, paratype, and epitype specimens are marked with HT, PT, and ET, respectively. New sequences obtained in this study are indicated in bold red.

Taxonomy

Meliola liboense **Y.Z. Feng & X.Y. Zeng, sp. nov.**
Index Fungorum: IF903772
Fig. 2

Etymology. in reference to the location (Libo County) where the specimen was collected.

Description. ***Parasitic*** on the surface of living leaves. Colonies 2–7 mm in diameter, hypophyllous, sometimes epiphyllous, black, dense to subdense. ***Hyphae*** superficial, straight to substraight, branched, septate, darker at septa, each cell 21–29 µm ($\bar{x}$ = 25 µm, n = 10) long, with dark brown setae. ***Hyphal setae*** 132–227 µm long, scattered, denser around ascomata, straight, 2 dentate, acute. Appressoria 13.2–16.7 × 8.1–12 µm ($\bar{x}$ = 15.2 × 9.7 µm, n = 20), 2-celled, brown, clavate, straight to curved, formed near septa, alternate to unilateral, antrorse. **Sexual morph:** ***Ascomata*** up to 120.8 µm in diameter, subdense, scattered, dark brown, globose to subglobose, outer wall rough, with a central ostiole and basal setae. ***Asci*** unitunicate, 2–3-spored, ovoid at young state, asci wall attenuated or broken when mature, without a fixed shape. ***Ascospores*** 39.4–47.6 × 12.6–17.9 µm ($\bar{x}$ = 42.7 × 16.1 µm, n = 22), hyaline when young, becoming dark brown when mature, 4 septate, constricted at the septa, rounded at both ends, smooth walled. Asexual morph not observed.

Material examined. CHINA • Guizhou Province, Libo County, 25°18'32"N, 107°56'10"E; on living leaves of *Lindera* sp.; 13 Jul. 2022; Yu–Wei Liu leg.; IFRD99008 (holotype) • ibid.; IFRD900-10 • ibid.; 8 Apr. 2022; IFRD900-9.

Notes. This collection is typical of *Meliola* in having hyphal setae and represents the first *Meliola* species with DNA sequence data from *Lindera*. There are 6% (541/576 nucleotides, 2 gaps) differences in the LSU region between *M. liboense* and *M. variaseta* and 5% (699/733 nucleotides, 6 gaps) differences in the LSU region between *M. liboense* and *M. vernalis*. However, the *M. vernalis* has larger ascomata (up to 275 µm) and longer hyphal setae (214.5–310 *vs.* 132–227 µm) than *M. liboense* (Pinho et al. 2012b). Although the original description of *M. linderae*, previously reported from the same host, is unavailable for detailed comparison, the present specimen differs clearly from known taxa based on distinct morphological traits and supported by phylogenetic analysis. Therefore, it is described herein as a new species, *M. liboense*.

Meliola lindericola **Y.Z. Feng & X.Y. Zeng, sp. nov.**
Index Fungorum: IF903770
Fig. 3

Etymology. in reference to the host species name.

Holotype. IFRD99007.

Description. ***Parasitic*** on the surface of living leaves. Colonies 10–12 mm in diameter, hypophyllous, sometimes epiphyllous, black, dense to subdense. ***Hyphae*** superficial, straight to substraight, branched, septate, darker at septa, each cell (18-) 21–45 µm ($\bar{x}$ = 40 µm, n = 20) long, with dark brown

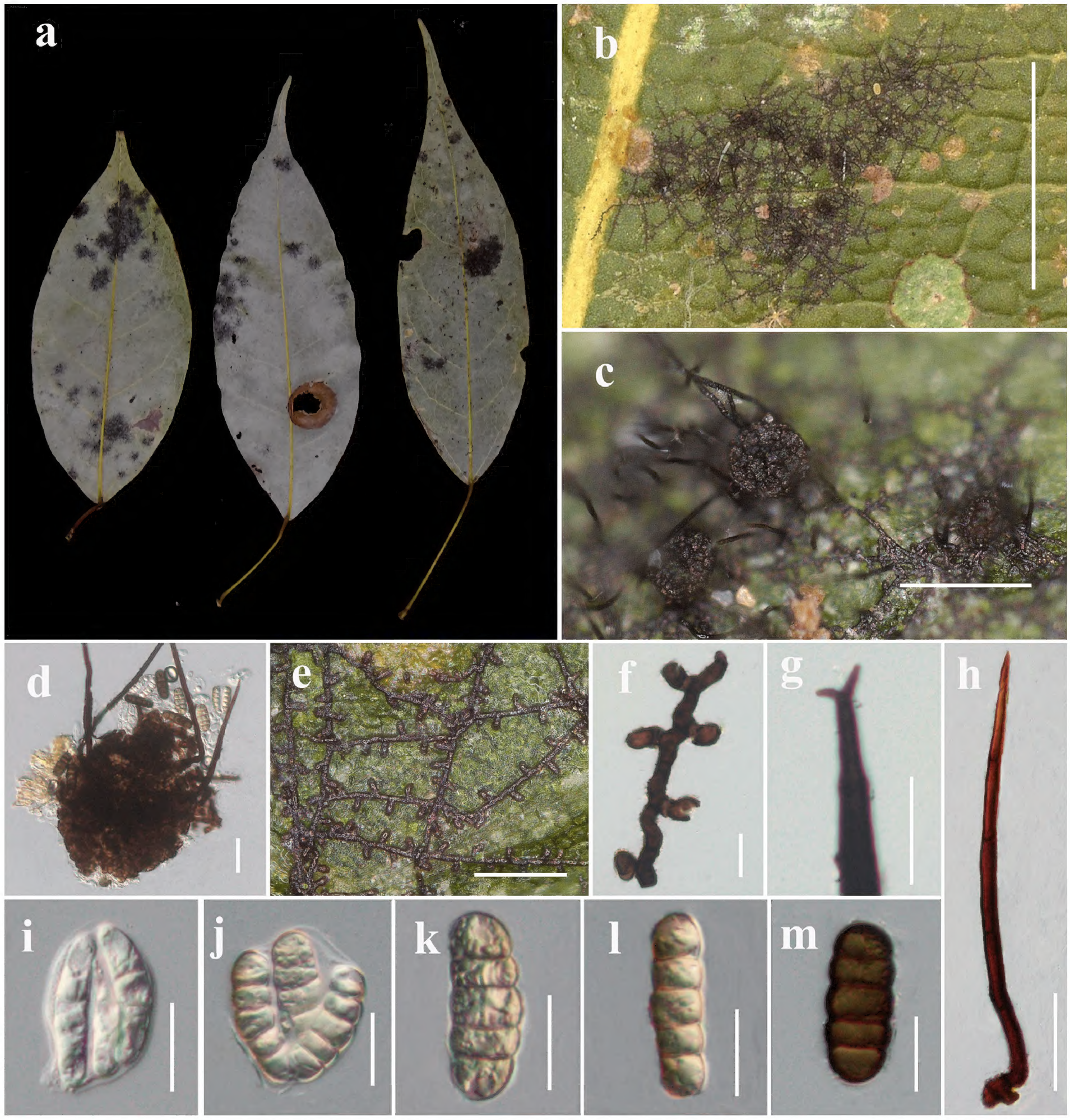

Figure 2. *Meliola liboense* (IFRD99008). **a.** Fresh leaf of *Lindera* sp.; **b.** Colonies on the leaf surface; **c.** Ascomata on the leaf surface; **d.** Ascomata; **e, f.** Reticulate hyphae with appressoria and phialides; **g.** Apex of setae; **h.** Hyphal setae; **i, j.** Asci; **k-m.** Ascospores. Scale bars: 2000 μm (**b**); 200 μm (**c**); 50 μm (**d**); 100 μm (**e, i-m**); 20 μm (**f, g**); 50 μm (**h**).

setae. ***Hyphal setae*** 121–162 μm long, scattered, denser around ascomata, straight, with obtuse tip. ***Asci*** unitunicate, 2–3-spored, ovoid at young state, asci wall attenuated or broken when mature, without a fixed shape. ***Appressoria*** 22.3–29.8 × 11–16.5 μm ($\bar{x}$ = 26.1 × 14 μm, n = 20), 2–celled, brown, clavate, straight to curved, formed near septa, alternate to unilateral, antrorse. **Sexual morph:** ***Ascomata*** up to 120 μm in diameter, subdense, scattered, dark brown, globose to subglobose, outer wall rough, with a central ostiole and basal setae. Asci not observed. ***Ascospores*** 21.3–25.5 × 6.8–9.3 μm ($\bar{x}$ = 21.7 × 8.2 μm, n = 30), hyaline ascospores 19.8–21.1 × 4.4–8.2 μm

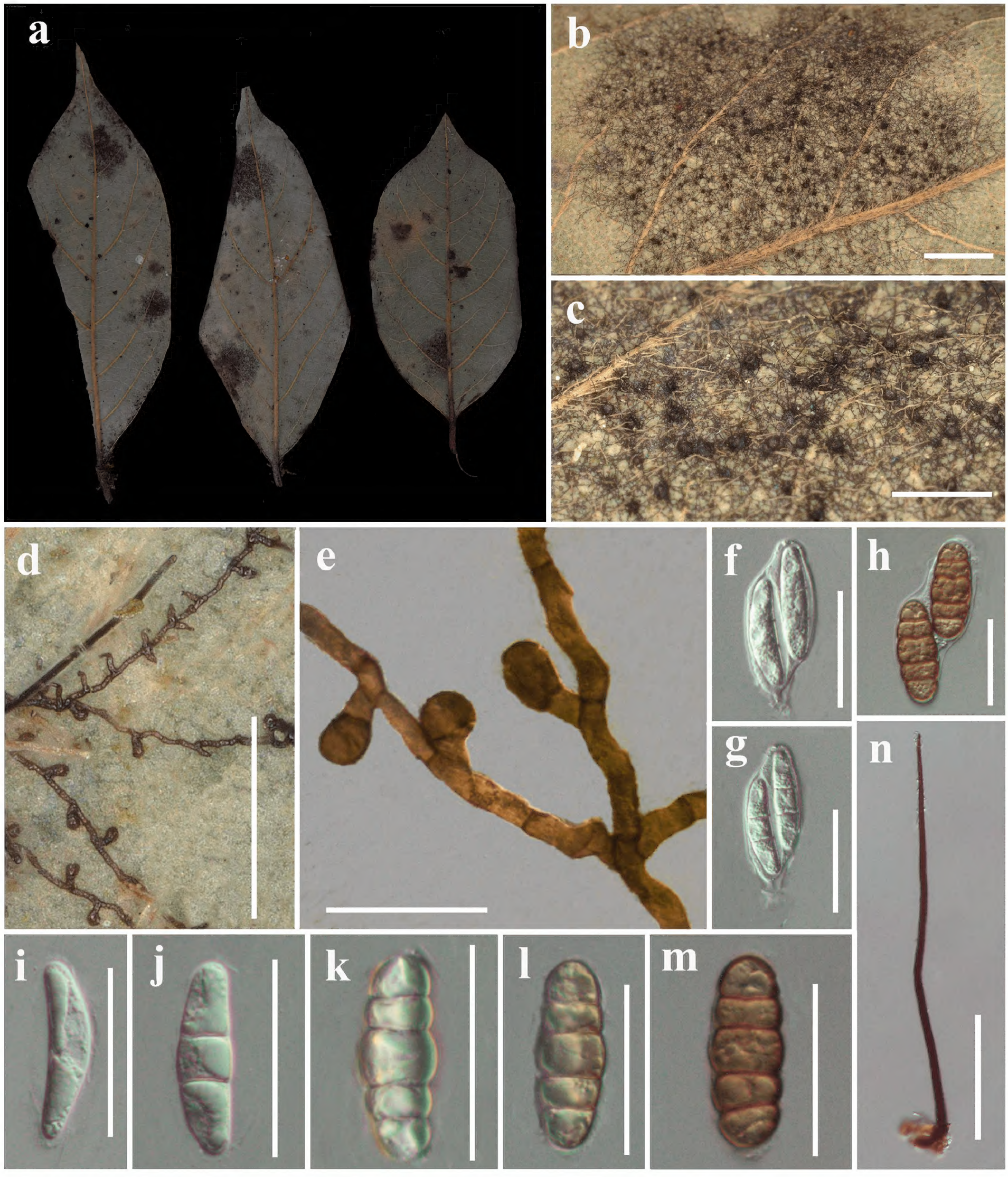

Figure 3. *Meliola lindericola* (IFRD99007). **a.** Fresh leaf of *Lindera* sp.; **b.** Colonies on the leaf surface; **c.** Ascomata on the leaf surface; **d, e.** Reticulate hyphae with appressoria and phialides; **f-h.** Asci from young to mature; **i-m.** Ascospores; **n.** Hyphal setae. Scale bars: 2000 μm (**b**); 1000 μm (**c**); 200 μm (**d**); 50 μm (**e**); 20 μm (**f-m**); 50 μm (**n**).

($\bar{x}$ = 20.6 × 5.7 μm, n = 6), cylindrical or oblong, hyaline when young, becoming dark brown when mature, 2–4-septate, constricted at the septa, rounded at both ends, smooth-walled. **Asexual morph:** Phialides 14.3–19.3 × 8.3–13 μm ($\bar{x}$ = 16.4 × 9.8 μm, n = 6), opposite to unilateral, few mixed with appressoria, ampulliform.

Material examined. CHINA • Guizhou Province, Libo County; 25°15'10"N, 107°44'6"E; on living leaves of *Lindera* sp.; 12 Jul. 2022; Yu-Wei Liu leg.; IFRD99007 (holotype).

Notes. This collection is typical of *Meliola* in having hyphal setae (Goos 1974). Phylogenetically, this new collection clusters with *M. pistaciicola*, but with 10% (186/203, 87/99 nucleotides, 5 gaps), 8% (773/837 nucleotides, 9 gaps), and 1% (994/999 nucleotides, no gaps) differences in ITS, LSU, and SSU regions, respectively. Moreover, *M. lindericola* is morphologically different in having shorter hyphae setae (162 *vs* 700 µm), smaller ascomata (120 *vs* 230 µm), ascospores (21.3–25.5 × 6.8–9.3 *vs* 43–55 × 17–25 µm), and phialides (14.3–19.3 × 8.3–13.0 *vs* 21–31 × 9–12 µm), and the phialides are opposite (Zeng et al. 2022). Besides, *M. lindericola* has larger appressoria (22.3–29.8 × 11.0–16.5 *vs* 13.2–16.7 × 8.1–12 µm), smaller ascospores (21.3–25.5 × 6.8–9.3 *vs* 39.4–47.6 × 12.6–17.9 µm), shorter setae (121–162 *vs* 132–227 µm), and an obtuse tip than *M. liboense*. Although the original description of *M. linderae* from the same host is unavailable for direct comparison, the distinct morphological features and molecular data obtained from our specimens support its recognition as a new species.

Discussion

Morphological and phylogenetic analyses of the collected specimens confirm the distinctiveness of *M. liboense* and *M. lindericola* as new species. Species delimitation was supported through an integrative approach combining detailed morphological examination, host information, and phylogenetic analysis. Both new species form independent lineages in the phylogenetic trees and show consistent differences from closely related taxa, confirming their recognition as distinct species. These findings enrich the known species diversity of *Meliola* and provide valuable molecular data for future studies within the family.

Currently, the classification of the genus *Meliola* still follows the system proposed by Hansford (1961), which is primarily based on host specificity and morphological characteristics. Since most species in Meliolaceae are known only from their sexual morphs, species delimitation heavily relies on traits such as the length and apical morphology of setae; the arrangement and dimensions of appressoria and phialides; the size of the ascomata; and the size and number of septa in ascospores. For example, *M. acaciae* and *M. peruiferi* can be distinguished based on differences in colony position, ascomata size, the arrangement of phialides and appressoria, and the apical morphology of setae (Zeng et al. 2022), while *M. hosagoudarii* and *M. litseicola* differ notably in the size and shape of ascospores (Song et al. 2002). Additionally, *M. pistaciicola* and *M. clerodendri-infortunati* are differentiated in that the latter has epiphyllous colonies, shorter hyphal cells, smaller appressoria and ascomata, larger ascospores, and simple, acute setae (Zeng et al. 2022). However, morphological characteristics often exhibit limited interspecific variation, and the considerable species diversity within the family further complicates accurate and efficient identification through traditional taxonomic methods.

In this study, only published sequences were included in the phylogenetic analyses to ensure data reliability. To avoid introducing systematic bias, *Endomeliola dingleyae* was excluded from the ingroup, as its phylogenetic position is markedly distant from other members of Meliolaceae. Zeng et al. (2022)

found that *Meliola* has traditionally been defined by the presence of setae, but it has diverged into two distinct phylogenetic lineages through co-evolution with angiosperms. Preliminary analyses suggest that this divergence may be related to the density of setae surrounding the ascomata. In addition, *Asteridiella* was revealed to be polyphyletic; phylogenetic analysis placed *Asteridiella obesa* within the clade of *Meliola* (Zeng et al. 2022). These findings imply that certain morphological characteristics, such as setal position, may not consistently correspond with phylogenetic relationships, indicating that the current morphology-based classification system may require further refinement. Although molecular sequence data for Meliolaceae are available in NCBI, some lack corresponding publications (e.g., KR706162, KC618648). Future taxonomic studies should combine molecular phylogenetic analyses with a critical reassessment of morphological traits to establish a more natural and robust classification for the family.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

Funding

This study was funded by the National Natural Science Foundation of China (nos. 32400013, 32060012, 32472527), Guizhou Provincial Basic Research Program (Natural Science) ZK[2023] general 087, and the Natural Science Special Research Fund of Guizhou University, Special Post (Gui Da Ling Jun He Zi [2024] 07).

Author contributions

Conceptualization: X.Y. Zeng, Y.W. Liu and Y.Z. Feng; methodology: X.Y. Zeng, Y.Z. Feng and Y.W. Liu; investigation: Y.W. Liu and Y.Z. Feng; data curation: X.L. Deng, T. Yang, J. P. Sun; formal analysis:Y. Z. Feng; funding acquisition: T. C. Wen, X. Y. Zeng and F. Q. Liu; writing – original draft: Y. Z. Feng; writing – review and editing: Y. Z. Feng, X.Y. Zeng, S. Hongsanan and E. Tarafder.

Author ORCIDs

Yuzhe Feng https://orcid.org/0009-0000-8832-7355
Yuwei Liu https://orcid.org/0000-0002-0346-149X
Sinang Hongsanan https://orcid.org/0000-0003-0550-3152
Entaj Tarafder https://orcid.org/0000-0002-3680-3433
Ting-Chi Wen https://orcid.org/0000-0003-1744-5869
Xiang-Yu Zeng https://orcid.org/0000-0003-1341-1004
Fengquan Liu https://orcid.org/0000-0001-9325-1500

Data availability

All of the data that support the findings of this study are available in the main text.

References

Beeli M (1920) Note sur le genre *Meliola* Fr. Espèces et variétés nouvelles récoltées au Congo. Bulletin du Jardin botanique de l'État a Bruxelles 7: 89–160. https://doi.org/10.2307/3666520

Bermúdez-Cova MA, Cruz-Laufer AJ, Piepenbring M (2022) Hyperparasitic fungi on black mildews (Meliolales, Ascomycota): Hidden fungal diversity in the tropics. Frontiers in Fungal Biology 3: 885279. https://doi.org/10.3389/ffunb.2022.885279

Bermúdez-Cova MA, Krauß A, Sanjur A, Tabé A, Hofmann TA, Yorou NS, Piepenbring M (2023) Diversity of hyperparasitic fungi on Meliolales (Sordariomycetes, Ascomycota): New species, records, and molecular data from Benin and Panama. Mycological Progress 22: 65. https://doi.org/10.1007/s11557-023-01913-5

Bhunjun CS, Niskanen T, Suwannarach N, Wannathes N, Chen YJ, McKenzie EHC, Maharachchikumbura SSN, Buyck B, Zhao CL, Fan YG, Zhang JY, Dissanayake AJ, Marasinghe DS, Jayawardena RS, Kumla J, Padamsee M, Chen YY, Liimatainen K, Ammirati JF, Phukhamsakda C, Liu JK, Phonrob W, Randrianjohany É, Hongsanan S, Cheewangkoon R, Bundhun D, Khuna S, Yu WJ, Deng LS, Lu YZ, Hyde KD, Lumyong S (2022) The numbers of fungi: Are the most speciose genera truly diverse? Fungal Diversity 114: 387–462. https://doi.org/10.1007/s13225-022-00501-4

Chethana KWT, Niranjan M, Dong W, Samarakoon MC, Bao DF, Calabon MS, Chaiwan N, Chuankid B, Dayarathne MC, de Sliva NI, Devadatha B, Dissanayake AJ, Goonasekara ID, Huanraluek N, Jayawardena RS, Karunarathna A, Luo ZL, Marasinghe DS, Ma XY, Norphanphoun C, Pem D, Perera RH, Rathnayaka AR, Raspé O, Samarakoon BC, Senwanna C, Sun YR, Tang X, Thiyagaraja V, Tennakoon DS, Zeng M, Zeng XY, Zhang JY, Zhang SN, Bulgakov TS, Camporesi E, Sarma VV, Wang Y, Bhat D, Hyde K (2021) AJOM new records and collections of fungi: 101–150. Asian Journal of Mycology 4: 113–260. https://doi.org/10.5943/ajom/4/1/8

Fries EM (1830) Eclogae fungorum, praecipue ex herbariis germanorum de scriptorum. Linnaea 5: 497–553.

Gaillard A (1892) Le genre *Meliola*. Bulletin de la Societe Mycologique de France 8: 176–188.

Goos R (1974) A scanning electron microscope and in vitro study of *Meliola palmicola*. The Proceedings of the Iowa Academy of Science 81: 23–27. https://scholarworks.uni.edu/pias/vol81/iss1/10

Goos R (1978) Field and laboratory studies of meliolaceous fungi in Hawaii. Mycologia 70: 995–1006. https://doi.org/10.1080/00275514.1978.12020315

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) JOURNAL ARTICLE New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology 59: 307–321. https://doi.org/10.1093/sysbio/syq010

Hall TA (1999) BioEdit: A user–friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hansford CG (1961) The Meliolineae: A monograph. Beihefte zur Sydowia 2: 1–806.

Hansford CG (1963) The Meliolineae Supplement. Sydowia 16: 302–323.

Hoang DT, Chernomor O, Haeseler Av, Minh BQ, Vinh LS (2017) UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution 35(2): 518–522. https://doi.org/10.1093/molbev/msx281

Hongsanan S, Tian Q, Peršoh D, Zeng XY, Hyde KD, Chomnunti P, Boonmee S, Bahkali AH, Wen TC (2015) Meliolales. Fungal Diversity 74: 91–141. https://doi.org/10.1007/s13225-015-0344-7

Hosagoudar VB (2008) Meliolales of India. Botanical Survey of India.

Hosagoudar VB (2013) Meliolales of India-Volume III. Journal of Threatened Taxa 5: 3993–4068. https://doi.org/10.11609/JoTT.o3307.3993-4068

Hyde KD, Hongsanan S, Jeewon R, Bhat DJ, McKenzie EHC, Jones EBG, Phookamsak R, Ariyawansa HA, Boonmee S, Zhao Q, Abdel-Aziz FA, Abdel-Wahab MA, Banmai S, Chomnunti P, Cui B-K, et al. (2016) Fungal diversity notes 367–490: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80: 1–270. https://doi.org/10.1007/s13225-016-0373-x

Index Fungorum (2025) Index Fungorum. http://www.indexfungorum.org/Names/Names.asp [accessed 21 April 2025]

Kalyaanamoorthy S, Minh BQ, Wong TKF, Haeseler Av, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Khan MB, Zeng XY, Wahab A, Sinang H, Huang SK, Wei DP, Liu ZH, Kang JC, He ZJ, Zhang X, Peng XC, Wen TC (2025) Host range variability and geographic distribution of *Meliola* species (Meliolales). Phytotaxa 689: 213–222. https://doi.org/10.11646/phytotaxa.689.2.4

Kress WJ, Erickson DL, Jones FA, Swenson NG, Perez R, Sanjur O, Bermingham E (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proceedings of the National Academy of Sciences of the United States of America 106: 18621–18626. https://doi.org/10.1073/pnas.0909820106

Lumbsch HT, Huhndorf SM (2010) Myconet volume 14. Part one. Outline of Ascomycota—2009. Part two. Notes on ascomycete systematics. Nos. 4751–5113. Fieldiana. Life and Earth Sciences: 1–64. https://doi.org/10.3158/1557.1

Marasinghe DS, Boonmee S, Hyde KD, Xie N, Hongsanan S (2020) Morpho-molecular analysis reveals *Appendiculella viticis* sp. nov. (Meliolaceae). Phytotaxa 454: 45–54. https://doi.org/10.11646/phytotaxa.454.1.4

Muhammad I, Nasir M, Ahmad NI, Muhammad S (2017) Biochemical and molecular analysis of laccase enzyme in saprobic fungus; *Sordaria fimicola*. International Journal of Agriculture and Biology 19: 204–210. https://doi.org/10.17957/IJAB/15.0265

Nannfeldt JA (1932) Studien uder die morphologie und systematik der nicht-lichenisierten, inoperculaten Discomyceten. Nova Acta Regiae Societatis Scientiarum Upsaliensis. Ser IV 69: 1–368.

Nguyen L-T, Schmidt HA, Haeseler Av, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. https://doi.org/10.1093/molbev/msu300

Old KM, Wingfield MJ, Yuan ZQ (2003) A manual of diseases of Eucalyptus in South-East Asia. Cifor.

Pinho DB, Firmino AL, Ferreira-Junior WG, Pereira OL (2012a) An efficient protocol for DNA extraction from Meliolales and the description of *Meliola centellae* sp. nov. Mycotaxon 122: 333–345. https://doi.org/10.5248/122.333

Pinho DB, Pereira OL, Firmino AL, Silva M, Ferreira-Junior WG, Barreto RW (2012b) New Meliolaceae from the Brazilian Atlantic forest 1. Species on hosts in the families Asteraceae, Burseraceae, Euphorbiaceae, Fabaceae and Sapindaceae. Mycologia 104: 121–137. https://doi.org/10.3852/10-260

Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of Gliocladium analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98: 625–634. https://doi.org/10.1016/S0953-7562(09)80409-7

Rodríguez Justavino D, Piepenbring M (2007) Two new species of *Appendiculella* (Meliolaceae) from Panama. Mycologia 99: 544–552. https://doi.org/10.1080/15572536.2007.11832548

Rodríguez Justavino D, Kirschner R, Piepenbring M (2015) New species and new records of Meliolaceae from Panama. Fungal Diversity 70: 73–84. https://doi.org/10.1007/s13225-014-0292-7

Ronquist F, Teslenko M, Mark Pvd, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Song B, Li TH, Goos R (2002) Three new species of the genus *Meliola* from China. Mycotaxon 84: 79–82.

Stevens FL (1927) The Meliolineae I. Annales Mycologici 25: 405–469.

Stevens FL (1928) The Meliolineae II. Annales Mycologici 26: 156–383.

Thite A (1975) Ascospore germination in *Meliola jasminicola*. Indian Phytopathology 28: 94–96.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zeng XY, Zhao JJ, Hongsanan S, Chomnunti P, Boonmee S, Wen TC (2017) A checklist for identifying Meliolales species. Mycosphere: Journal of Fungal Biology 8: 218–359. https://doi.org/10.5943/mycosphere/8/1/16

Zeng XY, Jeewon R, Wen TC, Hongsanan S, Boonmee S, Hyde KD (2018) Simplified and efficient DNA extraction protocol for Meliolaceae specimens. Mycological Progress 17: 403–415. https://doi.org/10.1007/s11557-017-1361-6

Zeng XY, Jeewon R, Hongsanan S, Hyde KD, Wen TC (2022) Unravelling evolutionary relationships between epifoliar Meliolaceae and angiosperms. Journal of Systematics and Evolution 60: 23–42. https://doi.org/10.1111/jse.12643

Zeng XY, Tan TJ, Tian FH, Wang Y, Wen T (2023) OFPT: A one-stop software for fungal phylogeny. Mycosphere: Journal of Fungal Biology 14: 1730–1741. https://doi.org/10.5943/mycosphere/14/1/20